PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 08 June 2000 (08.06.00)	
International application No. PCT/SE99/01784	**Applicant's or agent's file reference** 53371-56822
International filing date (day/month/year) 06 October 1999 (06.10.99)	**Priority date** (day/month/year) 06 October 1998 (06.10.98)
Applicant ZAPHIROPOULOS, Peter, G. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 May 2000 (05.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
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Replaced by Article 34 Amend

PATENT COOPERATION TREATY

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RECD 24 JAN 2001

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

15

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
53371-56822		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE99/01784	06.10.1999	06.10.1998
International Patent Classification (IPC) or national classification and IPC7		
A61K 48/00, C07K 14/705, A61K 38/17, G01N 33/68		
Applicant		
Karolinska Innovations AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.05.2000	12.01.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Patrick Andersson/EÖ Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1998)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/01784

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-23 , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☒ the claims:
pages _____ , as originally filed
pages _____ , as amended (together with any statement) under article 19
pages _____ , filed with the demand
pages 1,2 , filed with the letter of 15.12.2000

☐ the drawings:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

☐ the sequence listing part of the description:
pages _____ , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheet/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/01784

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 7 (partially), 8-10 and 16 completely

because:

☒ the said international application, or the said claims Nos. 7 (partially) and 16 completely relate to the following subject matter which does not require an international preliminary examination *(specify)*:

Claims 7 partially 16 completely relate to methods of treatment of the human or animal body by therapy/ diagnostic methods practised on the human or animal body See PCT Rule. 67.1.(iv).

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 8-10 are so unclear that no meaningful opinion could be formed *(specify)*:

Claim 8 relates to a screening method, it is not clear from this claim or the description how the polypeptide is used to achieve the screening. Claim 9 relates to a method of synthesis a modified drug, it is not clear from the description or the claim how the protein is used. Claim 10 relates to a drug identified by any of the methods in 8 or 9. Thus, claims 8-10 is considered to fail to comply with PCT-article 6 and have not been searched. Moreover, claim 15 concerns a drug per se, the drug may be a known substance.

.../...

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: III

The description or the claims does not give any indication on that any unknown substance(s) that are preferred. For this reason, a search or a statement concerning novelty and an inventive step can not be made for claim 10.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE99/01784

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5-7, 11-15	YES
	Claims	1-4, 17	NO
Inventive step (IS)	Claims		YES
	Claims	1-7, 11-15, 17	NO
Industrial applicability (IA)	Claims	1-7, 11-15, 17	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The claimed invention relates to nucleic acids, proteins participating in the human PTCH/SHH pathway as well as related vectors, cells, antibodies, methods and uses.

The following document is considered relevant:

D1) Motoyama J et al., ""Ptch2, a second mouse Patched gene is co-expressed with sonic hedgehog"

D1 discloses a mouse patched gene and its amino acid sequence having 91% homology in an 1140 amino acid overlap. The expression is analysed with RNA probes. In the present description a comparison between the sequence of D1 and the sequence claimed is made; the differences between the sequences are pointed out and it is speculated what effect these differences may have. However, the difference in function between the two has not been shown. Therefore, the expression "a variant thereof capable of participating in the human PTCH/SHH pathway" is unclear since a person skilled in the art get no information on what is covered e.g. by experiments showing unique functions of the claimed protein. The protein/nucleic acid of D1 would interact(i.e. be capable of participating) in some way also in the human pathway and can therefore be regarded as a variant of SEQ ID NO: 1. Thus, the invention according claims 1-4 and 17 lacks novelty.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

Nucleic acids hybridising to a nucleic acid, vectors, recombinant cells, antibodies, kits are all features obvious to a person skilled in the art; whether claims 11-15 involves an inventive step depends on if the claims the relate to are inventive.
Thus the invention according to claims 11-15 is novel and industrially applicable, but is not considered to involve an inventive step.

D1 discloses that the patched gene is involved in cancer, that the gene or its product can be used in some way in a medicament or diagnoses is considered obvious to a person skilled in the art. The present application seems not disclose any specific uses in medicament or diagnoses (e.g. a link between a mutation in the patched and a medical state) that goes beyond what a person skilled in the art would expect in view of D1. Thus the invention according to claims 5-7 is novel but not considered to involve an inventive step.

CLAIMS

1. An isolated human protein or an analogue or variant thereof capable of participating in the human PTCH/SHH pathway during embryonic development and/or carcinogenesis comprising at least about 1040 amino acids of SEQ ID NO: 1.
2. A protein according to claim 1, which is essentially comprised of SEQ ID NO: 1.
3. A nucleic acid encoding a protein according to any one of claims 1 and 2.
4. A nucleic acid encoding a protein according to claim 3 comprising at least about 3094 bases of SEQ ID NO: 2.
5. An isolated genomic nucleic acid comprising parts or all of SEQ ID NO: 5.
6. An isolated variant of the nucleic acid according to claim 5.
7. A nucleic acid having the sequence of a splicing variant selected from the group of variants defined in Figure 2B and SEQ ID NO:3 and SEQ ID NO:4.
8. An isolated nucleic acid capable of specifically hybridising to a nucleic acid according to any one of claims 3-7.
9. A protein or polypeptide encoded by a nucleic acid according to claim 7.
10. A protein according to claim 1 or 2, a nucleic acid according to any one of claims 2-8 or or a polypeptide according to claim 9, for use as a medicament.
11. Use of a protein according to claim 1 or 2, a nucleic acid according to any one of claims 2-8 or a polypeptide according to claim 9 in the manufacture of a medicament for the treatment of a condition involving tumors, such as BSS.
12. A method of in vitro or in vivo diagnosis, wherein a protein according to claim 1 or 2, a nucleic acid according to any one of claims 2-8 or a polypeptide according to claim 9 is used.
13. A method of screening wherein a library of suitable candidate compounds is screened for modified drugs using a protein according to claim 1 or 2 or a polypeptide according to claim 9 as a lead compound.
14. A method of synthesis of a modified drug, wherein a protein according to claim 1 or 2, or a protein or polypeptide according to claim 9, is used.
15. A modified drug identified by the method according to claim 13 or synthesized according to claim 14.

16. A vector comprising a nucleic acid according to any one of claim 3-8.

17. A recombinant cell comprising a vector according to claim 16.

18. An antibody which specifically binds to a protein or polypeptide according to claim 1, 2 or 9.

19. A recombinant cell expressing an antibody according to claim 18.

20. A kit for the detection of a human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid according to any one of claims 3-8, a polypeptide or protein according to claim 1, 2 or 9 or an antibody according to claim 18.

21. Use of a nucleic acid according to any one of claims 3-7 in gene therapy.

22. Use of nucleic acid according to any one of claims 3-7 as a probe, a primer or a diagnostic reagent.

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(54) Title: A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY

(57) Abstract

The present invention relates to a novel human patched–like gene (PTCH2), which for the first time has been cloned and sequenced. Several alternatively spliced mRNA forms of PTCH2 have been identified, including transcripts lacking segments thought to be involved in sonic hedgehog (SHH) binding and mRNAs with differentially defined 3' terminal exons. Further, the invention also relates to the protein encoded by the present PTCH2 as well as to functional analogues and variants thereof.

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A NOVEL COMPONENT IN THE HEDGEHOG SIGNALLING PATHWAY

Technical field

The present invention relates to novel molecules, such as proteins, polypeptides and nucleotides, involved in the hedgehog signalling pathway with putative involvement in embryonic development and carcinogenesis. The invention also relates to various novel advantageous uses of the molecules according to the invention, e.g. in diagnosis and therapy.

Background

In the study of the development of cells, fruit flies have extensively been used as a model, as they are less complex than mammalian cells.

Pattern formation takes place through a series of logical steps, reiterated many times during the development of an organism. Viewed from a broader evolutionary perspective, across species, the same sort of reiterative pattern formations are seen. The central dogma of pattern formation has been described (Lawrence and Struhl, 1996). Three interlocking and overlapping steps are defined. Firstly, positional information in the form of morphogen gradients allocate cells into non-overlapping sets, each set founding a compartment. Secondly, each of these compartments acquire a genetic address, as a result of the function of active "selector" genes, that specify cell fate within a compartment and also instruct cells and their descendents how to communicate with cells in neighboring compartments. The third step involves interactions between cells in adjacent compartments, initiating new morphogen gradients, which directly organize the pattern.

Taking these steps in greater detail, one finds the first step in patterning to be the definition of sets of cells in each primordium. Cells are allocated according to their positions with respect to both dorsoventral and anterior/posterior axes by morphogen gradients. Allocation of cells in the dorsoventral axis constitutes the germ layers, such as mesoderm or neurectoderm.

In segmentation, the second step (the specification of cell fate in each compartment) is carried out by the gene *engrailed* and elements of the bithorax complex. *Engrailed* defines anterior and posterior compartments both in segmentation and in limb specification.

The third step in pattern formation, secretion of morphogens, functions to differentiate patterns within compartments (and thereby establish segment polarity). Initially, all cells within a compartment are equipotent, but they become diversified to form pattern. Pattern formation depends on gradients of morphogens, gradients initiated along compartment boundaries. Such gradients are established by a short-range signal induced in all the cells of the compartment in which the above mentioned selector gene *engrailed* is active. For segment polarity, this signal is Hedgehog. In the adjacent compartment the selector gene is inactive, ensuring that the cells are sensitive to the signal. The Hedgehog signal range is probably only a few rows of cells wide; responding cells become a linear source of a long-range morphogen, that diffuses outward in all directions. There are three known Hedgehogs, Sonic (SHH), Indian (IHH) and Desert (DHH). The proteins they encode can substitute each for each other, but in wildtype animals, their distinct distributions result in unique activities. SHH controls the polarity of limb growth, directs the development of neurons in the ventral neural tube and patterns somities. IHH controls endochondral bone development and DHH is necessary for spermiogenesis. Vertebrate hedgehog genes are expressed in many other tissues, including the peripheral nervous system, brain, lung, liver, kidney, tooth primordia, genitalia and hindgut and foregut endoderm.

Thus, segment polarity genes have been identified in flies as mutations, which change the pattern of structures of the body segments. Mutations in these genes cause animals to develop the changed patterns on the surfaces of body segments, the changes affecting the pattern along the head to tail axis. For example, mutations in the gene *patched* cause each body segment to develop without the normal structures in the center of each segment. Instead there is a mirror image of the pattern normally found in the anterior segment. Thus, cells in the center of the segment make the

wrong structures, and point them in the wrong direction with reference to the over all head-to-tail polarity of the animal.

About sixteen genes in the class are known. The encoded proteins include kinases, transcription factors, a cell junction protein, two secreted proteins called wingless (WG) and the above mentioned Hedgehog (HH), a single transmembrane protein called patched (PTC) and some novel proteins not related to any known protein. All of these proteins are beleived to work together in signaling pathways that inform cells about their neighbors in order to set cell fates and polarities.

PTC has been proposed as a receptor for HH protein based on genetic experiments in flies. A model for the relationship is that PTC acts through a largely unknown pathway to inactivate both its own transcription and the transcription of the *wingless* segment polarity gene. This model proposes that HH protein, secreted from adjacent cells, binds to the PTC receptor, inactivates it and thereby prevents PTC from turning off its own transcription or that of *wingless*. A number of experiments have shown coordinate events between PTC and HH.

Human *patched* gene (PTCH) was recently identified as the gene responsible for the nevoid basal cell carcinoma syndrome (NBCCS), also known as the Gorlin Syndrome, which is an autosomal dominant disorder that predisposes to both cancer and developmental defects (Gorlin (1995) *Dermatologic Clinics* 13:113-125) characterized by multiple basal cell carcinomas (BCCs), medulloblastomas and ovarian fibromas as well as numerous developmental anomalities (Hahn, H., Wicking, C., Zaphiropoulos, P.G., Gailani, M.R., Shanley, S., Chidambaram, A., Vorechovsky, I., Holmberg, E., Undén, A.B., Gillies, S., Negus, K., Smyth, I., Pressman, C., Leffell, D.J., Gerrard, B., Goldstein, A.M., Dean, M., Toftgård, R., Chenevix-Trench, G., Wainright, B. and Bale, A.E. (1996): "Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome", Cell **85**, 841-851; and Johnson, R.L., Rothman, A.L., Xie, J., Goodrich, L.V., Bare, J.W., Bonifas, J.M., Quinn, A:G., Myers, R.M., Cox, D.R., Epstein, E.H. Jr and Scott, M.P.

(1996): "Human homolog of patched, a candidate gene for the basal cell nevus syndrome", Science **272**, 1668-1671). PTCH codes for a membrane receptor of the autolytically cleaved (protein spliced), amino terminal domain of *sonic hedgehog* (SHH) (Mariago, V., Davey, R.A., Zuo, Y., Cunningham, J.M. and Tabin, C.J. (1996): "Biochemical evidence that patched is the Hedgehog receptor", Nature **384**, 176-179; and Stone, D.M., Hynes, M., Armanini, M., Swanson, T.A., Gu, Q., Johnson, R.L., Scott, M.P., Pennica, D., Goddard, A., Phillips, H., Noll, M., Hooper, J.E., de Sauvage, F. and Rosenthal, A. (1996): "The tumor-suppressor gene patched encodes a candidate receptor for Sonic hedgehog", Nature **384**, 129-134). In the non-signalling state, PTCH is thought to inhibit the consecutive signalling of another membrane protein, *smoothened* (SMO), however binding of SHH to PTCH releives this inhibition (Goodrich, L.V., Milenkovic, L., Higgins, K.M. and Scott, M.P. (1997): "Altered neural cell fates and medullablastom in mouse patched mutants", Science **277**, 1109-1113). This cascade of signalling events, best characterized in Drosophila, also involves a number of intracellular components including *fused* (a serine threonine kinase), *suppressor of fused*, *costal 2*, and *cubitus interruptus* (Ruiz i Altaba, A.,: "Catching a Gli-mpse of Hedgehog" (1997) Cell **90**, 193-196). The latter is a transcription factor that positively regulates the expression of target genes which also include PTCH itself.

Mutations in the PTCH gene have been identified in both sporadic and familial BCCs (Gailani, M.R., Ståhle-Bäckdahl, M., Leffell, D.J., Glynn, M., Zaphiropoulos, P.G., Pressman, C., Undén, A.B., Dean, M., Brash, D. E., Bale, A.E. and Toftgård, R. (1996): "The role of human homologue of Drosophila patched in sporadic basal cell carcinomas" Nature Genet. **14**, 78-81). The lack of the normal PTCH protein in these cells allows the constitutive signalling of SMO to occur, resulting in the accumulation of mutant PTCH mRNAs (Undén, B. A., Zaphiropolous, P.G., Bruce, K., Toftgård, R., and Ståhle-Bäckdahl, M. (1997): "Human patched (PTCH) mRNA is overexpressed consistently in tumor cells of both familial and sporadic basal cell carcinoma", Cancer Res. **57**, 2336-2340).

WO 96/11260 discloses the isolation of *patched* genes and the use of the PTC protein to identify ligands, other than the established ligand Hedgehog, that bind thereto.

However, there is still a need of a further understanding of the SHH/PTCH cell signalling, which may be provided by disclosure of further genes, peptides and proteins involved therein.

Summary of the invention

The present invention provides a significant step forward regarding the understanding of the above described pathway. By a combination of cDNA library and RACE analysis a novel human *patched*-like gene (PTCH2) has been cloned and sequenced. Several alternatively spliced mRNA forms of PTCH2 have been ideintified, including transcripts lacking segments thought to be involved in sonic hedgehog (SHH) binding and mRNAs with differentially defined 3' terminal exons. Accordingly, the invention relates to isolated such mRNAs as well as to cDNAs complementary thereto.

Brief description of the drawings

Figure 1 shows the genomic sequence of SEQ ID NO:5, wherein exons and introns are designated in the genomic sequence of the novel human patched 2 gene.

Figure 2A discloses an amino acid sequence comparison of the human PTCH2 (upper lines) and PTCH1 (lower lines) sequences.

Figure 2B is a representation of the alternative splicing events that result in different C-termini.

Figure 2C is a representation of the different variations of spliced transcripts encompassing exon 1 and exon 2 sequences.

Figure 3A is a dark-field photomicrograph of a BCC tumor hybridised with ^{35}S-labeled antisense probe showing abundant signal for PTCH1 mRNA (light grains) in all BCC tumor cells.

Figure 3B discloses PTCH2 mRNA overexpression in BCC and is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests.

Figure 3C is another BCC showing a strong PTCH2 mRNA signal in the periphery of the tumor nest (Tu), wheras no signal is detected in epidermis (Ep).

Figure 3D are sections of the same tumor (C) hybridised with the PTCH2 sense probe showed no signal.

Figure 3E shows immunoreactivity for Ki-67.

Figure 3F discloses how tumor nests under high power magnification demonstrate abundant PTCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow).

Definitions

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

The terms "isolated" "purified" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state.

The term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as sommonly used in a ELISA), biotin, dioxigenin, or haptens and proteins for which antisera or mono-

clonal antibodies are available (*e.g.*, the peptide of SEQ ID NO:1 can be made detectable, *e.g.*, by incorporating a radio-label into the peptide, and used to detect antibodies specifically reactive with the peptide).

As used herein a "nucleic acid probe" is defined as a nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation. As used herein, a probe may include natural (*i.e.* A, G, C, or T) or modified bases (7-deazaguanosine, inosine, *etc.*) In addition, the bases in a probe may be joined by a linkage other than a phosphodiester bond, so long as it does not interfere with hybridisation. Thus, for example, probes may be peptide nucleic acids in which the constituent bases are joined by peptide bonds rather that phosphodiester linkages. It will be understood by one of skill in the art that probes may bind target sequences lacking complete complementarity with the probe sequence depending upon the stringency of the hybridisation conditions. The probes are preferably directly labeled as with isotopes, chromophores, lumiphore, chromogens, or indirectly labeled such as with biotin to which a streptavidin complex may later bind. By assaying for the presence or absence of the probe, one can detect the presence or absence of the selct sequence or subsequence.

A "labeled nucleic acid probe" is a nucleic acid probe that is bound, either covalently, through a linker, or through ionic, van der Waals or hydrogen bonds to a label such that the presence of the probe may be detected by detecting the presence of the label bound to the probe.

The term "target nucleic acid" refers to a nucleic acid (often derived from a biological sample), to which a nucelic acid probe is designed to specifically hybridise. It is either the presence or absence of the target nucleic acid that is to be detected, or the amount of the target nucleic acid that is to be quantified. The target nucleic acid has a sequence that is complementary to the nucleic acid sequence of the corresponding probe directed to the target. The term target nucleic acid may refer to the specific

subsequence of a larger nucleic acid to which the probe is directed or to the ovarall sequence (*e.g.*, gene or mRNA) whose expression level it is desired to detect. The difference in usage will be apparent from context.

The term "recombinant" when used with reference to a cell, or nucleic acid, or vector, indicates that the cell, or nucleic acid, or vector, has been modified by the introduction of a heterologous nucleic acid or the alteration of a native nucleic acid, or that the cell is derived from a cell so modified.

The term "identical" in the context of two nucleic acids or polypeptide sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorith of Smith and Waterman (1981) *Adv. Appl. Math.* 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* USA 85: 2444, by computerized implementations of these algorithms (GAP, GESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI) or by inspection. The BLAST algorithm performs a statistical analysis of the similarity between two sequences; *see e.g.*, Karlin and Altschul (1993) Proc. *Nat'l Acad. Sci. USA* 90: 5873-5787.

The term "substantial identity" or "substantial similarity" in the context of a polypeptide indicates that a polypeptides comprises a sequence with at least 70% sequence identity to a reference sequence, or preferably 80%, or more preferably 85% sequence identity to the reference sequence, or most preferably 90% identity over a comparison window of about 10-20 amino acid residues. An indication that two polypeptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a polypeptide is substantially identical to a second polypeptide, for example, where the two peptides differ only by a conservative substitution.

An indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridise to each other under stringent conditions.

The phrase "hybridising specifically to", refers to the binding, duplexing, or hybridising of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular) DNA or RNA. The term "stringent conditions" refers to conditions under which a probe will hybridise to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridise specifically at higher temperatures. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point ™ for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridise to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the probes are occupies at equilibrium). Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for whort probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.

The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen).

A "chimeric antibody" is an antibody molecule in which (a) the constant region, or a portion thereof, is altered, replaced or exchanged so that the antigen binding site (variable region) is linked to a constant region of a different or altered class, effector function and/or species, or an entirely different molecule which confers new properties to the chimeric antibody, *e.g.*, an enzyme, toxin, hormone, growth factor, drug, *etc.*; or (b) the variable region, or a portion thereof, is altered, replaced or exchanged with a variable region having a different or altered antigen specificity.

The term "immunoassay" is an assay that utilizes an antibody to specifically bind an analyte. The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the analyte.

The phrases "specifically binds to a protein" or "specifically immunoreactive with", when referring to an antibody refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind preferentially to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antoibodies specifically immunoreactive with a protein. *See* harlow and Lane (1988) *Antibodies, A Laboratory Manual,* Cold Spring Harbour Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

A "gene product", as used herein, refers to a nucleic acid whose presence, absence, quantity, or nucleic acid sequence is indicative of a presence, absence, quantity, or nucleic acid composition of the gene. Gene products thus include, but are not limited to, and mRNA transcript acDNA reverse transcribed from an mRNA, and RNA

transcribed from that cDNA, a DNA amplified from the cDNA, an RNA transcribed from the amplified DNA or subsequences of any of these nucleic acids. Polypeptides expressed by the gene or subsequences thereof are also gene products. The particular type of gene product will be evident from the context of the usage of the term.

A "modified drug" means a compound, which retains the pharmaceutical properties of the original drug or active substance while the structure thereof has been modified. Further, encompassed by the term "drug" are also compounds useful in diagnostic methods by their specific binding properties.

Detailed description of the invention

In a first aspect, the present invention relates to an isolated human protein, or an analogue or a variant thereof, capable of participating in the human PTCH/SHH pathway during embryonic development and/or carcinogenesis, such as basal cell carcinoma. The novel protein according to the invention is encoded by a novel gene, which isolated nucleic acid is described in detail below and which is denoted *patched 2* (PTCH2) due to its similarities with *patched 1* (PTCH1). Accordingly, the protein according to the invention exhibits substantial differences in sequence and functions when compared to human PTCH1protein. The protein according to the invention is best characterized by its functions which when compared to human PTCH1 are similar but distinct therefrom in certain ways, more specifically disclosed below in the section "Results and discussion". The novel human PTCH2 protein according to the invention is also distinct from the previously isolated mouse PTCH2. Thus, in the preferred embodiment thereof, it comprises a substantial part of the amino acid sequence disclosed in SEQ ID NO: 1 and submitted to the GenBank under protein id no AAD17260.1. even though it is to be understood that the present invention encompasses any fragment, analogue or variant thereof exhibiting the biological functions of the PTCH2 protein disclosed herein. Thus, preferably, the present protein comprises at least about 1000, more preferably at least about

1040 and most preferably essentially all of the amino acids of the sequence denoted SEQ ID NO: 1, such as about 1100.

The proteins according to the invention are easily prepared by someone skilled in this field by recombinant DNA techniques using the molecules disclosed below or any synthetic method (*see e.g.* Barany and Merrifield, Solid-Phase Peptide synthesis, pp. 3-284 in The Peptides: Analysis, Synthesis, Biology, Vol. 2: Special Methods in Peptide synthesis, Part A, Merrifield *et al.*, J. Am. Chem. Soc., 2149-2156).

The present invention also relates to the use of the peptides, polypeptides and proteins disclosed herein as lead compounds in methods aimed at finding novel substances, i.e. modified drugs, such as substances exhibiting equivalent or even more advantageous properties than the lead compounds as such. Such modified drugs may also be designed by methods of combinatorial chemistry, wherein a structurally similar compound is specifically designed e.g. by aid of computers. Alternatively, the present modified drug is identidied by screening of a library of candidate compounds, e.g. using an antibody according to the invention. In the present context, it is to be understood that when such a modified drug has been identified, it is possible to produce it by any other suitable technique. The invention also relates to proteomic methods wherein the present molecules are used as well as to such a use *per se*.

A second aspect of the present invention is a nucleic acid encoding a protein, an analogue or a variant thereof as defined above, that is, the protein coding region of the novel human isolated PTCH2 gene. The PTCH2 gene is 57% identical to PTCH1 and 91% identical to the published mouse Ptch2 sequence (*see* Motoyama *et al.*, (1998), supra). Thus, preferably, the nucleic acid according to the present invention comprises at least about 3000 bases, more preferably at least about 3094 bases and most preferably essentially all of the sequence denoted SEQ ID NO: 2.

In a specific aspect, the present invention relates to the isolated human genomic PTCH2 nucleic acid comprising parts or all of the genomic sequence denoted SEQ ID NO: 5. In the disclosure of the genomic sequence shown in Fig 1, the exon/intron structure of the present gene is shown. Further to the exons shown therein, exon 12a and 12b has also been identified, as specifically defined by SEQ ID NO:3 and SEQ ID NO:4, respectively. Interestingly, there is a splice variant that joins exon 12a to a 3' segment of exon 12b with conservation of the intronic GT-AG dinucleotides. Exons 12a and 12b are not variants, but the actual exons of the gene identified by sequencing the corresponding genomic region. (Materials and methods were as discribed beloow). Accordingly, these findings show that PTCH2 has the same intron/exon structure organization as PTCH1. In another embodiment of this aspect, the present invention relates to a transcript that has skipped only one of the exons 9 and 10 defined in Fig 1. In an alternative embodiment, the transcript according to the invention has skipped both of exon 9 and 10. The splice variants of the present gene are discussed in more detail below in the section "Results", all of which are included within the scope of the present invention. This aspect of the invention advantageously enables design of suitable PCR primers, which in turn enables screening for mutations of all of the coding sections thereof, *e.g.* by SSCP analysis, sequencing, or any other suitable method known to someone skilled in this field. Thus, the novel human PTCH2 gene according to the invention has been localized by radiation hybrid mapping to chromosome 1p32-35 with D1S211 and WI-1404 as closest flanking markers and with an estimated localization 5.5cR from D1S443. This region is often lost by LOH in various different tumor types, such as neuroblastoma, melanoma, breast cancer, colon cancer *etc.* Accordingly, PTCH2 is a candidate for a tumor suppressor gene in this region and the present invention also encompass diagnostic methods based on this new disclosure.

To this chromosomal region, three cancer predisposition syndromes have also been mapped, namely, familial melanoma CMM1, modifier locus for familial adenomatous polyposis hMom1 and Michelin Tire Baby Syndrome. PTCH2 is further a candidate for the gene behind these heritary syndromes. The present molecules are the-

refore advantageously used in the context of these conditions, *e.g.* in therapy and/or diagnosis, such as in assays.

Further, the invention also relates to various PCR primers based on intronic sequences, allowing amplification of all coding sequence. Such primers are advantageously used for mutation screening.

Further, the present invention also relates to the any isolated nucleic acid capable of specifically hybridising to a nucleic acid according to the invention. In addition, the invention also relates to such an isolated nucleic acid which comprises one o more mutations compared to the genomic sequence as well as the use of the novel isolated nucleic acids, *e.g.* to identify mutations for diagnostic and/or therapeutic purposes.

Further embodiments of this aspect of the invention includes nucleic acid probes, *e.g.* DNA probes, labelled nucleic acids, cDNAs, RNAs *etc.*, that is, all gene products obtainable by someone skilled in this field based on the novel isolated human PTCH2 gene.

Another aspect of the invention is a nucleic acid corresponding to any one of the splicing variants disclosed in Figure 2B , a protein or polypeptide encoded thereof as well as various uses thereof.

As regards the preparation of nucleic acids according to the invention, any suitable recombinant DNA technique or synthetic method may be used. (For general laboratory procedures useful in this context, *see e.g.* Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 2nd ed., Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 153, Academic Press, Inc., San Diego, CA; Current Protocols in Molecular Biology, F.M. Ausbel *et al.*, eds., Current Protocols (1994)).

A further aspect of the present invention is a vector comprising a nucleic acid as defined above. Vectors are *e.g.* useful for transforming cells in vitro or in vivo to express the proteins and peptides according to the invention and may e.g be plasmids, viruses *etc.*

Another aspect of the invention is a recombinant cell, such as a eucaryotic, *e.g.* a mammalian cell, or a procaryotic cell, *e.g.* a bacteria, comprising a vector as defined above. Such cells may *e.g.* be used to monitor expression levels of the proteins and polypeptides according to the invention in a wide variety of contexts. For example, when the effects of a drug is to be determined, the drug will be administered to the transformed organism, tissue or cell. Accordingly, model systems including such cells are another aspect of the invention.

A further aspect of the invention is an antibody, such as a monoclonal or polyclonal antibody, which specifically binds to a protein or polypeptide according to the invention. An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable haeavy chain (V_H) refer to these light and heavy chains, respectively.

The invention also encompasses chimeric or other antibodies that binds the present proteins or polypeptides. Further, the invention also relates to the use of the present antibodies in assays. (In this context, *see e.g. Fundamental Immunology,* Third Edition, W.E. Paul, ed., Raven Press, N.Y. 1993).

Further, the invention also relates to a recombinant cell expressing an antibody according to the invention.

In general, prokaryotes can be used for cloning the DNA sequences encoding a human anti-PTCH2 immunoglobulin chain. *E. coli* is one prokaryotic host particularly useful for cloning the DNA sequences of the present invention. Microbes, such as yeast are also useful for expression. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase 2, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

Mammalian cells are a particularly preferred host for expressing nucleotide segments encoding immunoglobulins or fragments thereof (*see, e.g.* Winnacker, *From Genes to Clones,* VCH Publishers, N.Y., 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication , a promoter, an enhancer (Queen *et al.* (1986) *Immunol. Rev.* 89:49), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine pappillomavirus, and the like (*see, e.g.*, Co *et al.* (1992) *J. Immunol.* 1458: 1149).

An additional aspect of the present invention is a kit for the detection of a human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid, a polypeptide or a protein or an antibody according to the invention. Further suitable components of such a kit are easily determined by someone skilled in this field as are the conditions for the use thereof.

Further, the invention also realtes to the use of a nucleic acid selected from the group consisting of SEQ ID NOS: 2-4 and SEQ ID NO: 5 in gene therapy. In addition to said specifically disclosed sequences, any one of the herein disclosed exons may be used to this end.For a review of gene therapy procedures, *see* Anderson, *Science* (1992) 256:808-813; Nabel and Felgner (1993) *TIBTECH* 11: 211-217; Mitani and Caskey (1993) *TIBTECH* 11: 162-166; Mulligan (1993) *Science* 926-932; Dillon (1993) *TIBTECH* 11: 167-175; Miller (1992) *Nature* 357: 455-460; Van Brunt (1988) *Biotechnology* 6(10): 1149-1154; Vigne (1995) *Restorative Neurology and Neuroscience* 8: 35-36; Kremer and Perricaudet (1995) *British Medical Bulletin* 51(1) 31-44; Haddada *et al.* (1995) in *Current Topics in Microbiology and Immunology* Doerfler and Böhm (eds) Springer-Verlag, Heidelberg Germany; and Yu *et al., Gene Theraphy*(1994) 1:13-26.

Delivery of the gene or genetic material into the cell is the first critical step in gene therapy treatment of disease. A large number of delivery methods are well known to those of skill in the art. Such methods include, for example liposome-based gene delivery (Debs and Zhu (1993) WO 93/24640; Mannino and Gould-Fogerite (1988) *BioTechniques* 6(7): 682-691; Rose U.S. Pat No. 5,279,833; Brigham (1991) WO 91/06309; and Felgner *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 7413-7414), and replication-defective retroviral vectors harboring a therapeutic polynucleotide uence as part of the retroviral genome (*see, e.g.*, Miller *et al.* (1990) *Mol. Cell. Biol.* 10:4239 (1990; Kolberg (1992) *J. NIH Res.* 4:43, and Cornetta *et al. Hum. Gene Ther.* 2:215 (1991)). Widely used retroviral vectors include those based upon nurine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof. *See, e.g.*, Buchscher *et al.* (1992) *J. Virol.* 66 (5) 2731-2739; Johann *et al.* (1992) *J. Virol.* 66 (5):1635-1640 (1992); Sommerfelt *et al.*, (1990) *Virol.* 176:58-59; Wilson *et al.* (1989) *J. Virol.* 63:2374-2378; Miller *et al., J Virol.* 65:2220-2224 (1991); Wong-Staal *et al.*, PCT/US94/05700, and Rosenburg and Fauci (1993) in *Fundamental Immunology, Third Edition* Paul (ed) Raven Press, Ltd., New York and the references therein, and Yu *et al., Gene Therapy* (1994) supra).

The present invention may also be used in the pharmaceutical industry. For example, it will provide information that eventually may enable cells from fetal tissue, which may the be transplanted into patients suffering from *e.g.* Parkinson's disease or cancer, such as BCC. (For a brief review of methods of drug delivery, *see* Langer 249:1 527-1533 (1990), Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985) *etc.*)

Detailed description of the drawings

Figure 1 shows the genomic sequence of SEQ ID NO:5, wherein exons and introns are designated in the genomic sequence of the present human patched 2 gene. However, exons 12a and 12b discussed above are not specifically shown in Figure 1, but is instead disclosed as the separate sequences SEQ ID NO:3 and SEQ ID NO:4, respectively. Figure 2A discloses an amino acid sequence comparison of the human PTCH2 (upper lines) and PTCH1 (lower lines) sequences. Vertical lines indicate identical amino acids, while dots similar amino acids. The PTCH2 sequence presented is composed of the original cDNA clones and of the products of the 5' RACE analysis.

Figure 2B is a representation of the alternative splicing events that result in different C-termini. In the parotid gland and the colon, the penultimate and the last exon are canonically joined together. In fetal brain however the penultimate exon with part of the 3' intron functions as the terminal exon. The intronic sequence is shown by small letters with the flanking exonic by capital letters. Above the nucleotide sequence, the deduced amino acid sequence is shown, and below is the corresponding sequence of the mouse Ptch2. The conserved intronic dinucleotides are shown by bold letters and the termination signals are indicated by asterisks. Note the absence of conservation of the position of the termination codons between the mouse and human PTCH2 sequences. The putative polyadenylation signals are also shown in this diagram. The genomic organization was obtained by analyzing BAC clones encompassing the PTCH2 gene.

Figure 2C is a representation of the different variations of spliced transcripts encompassing exon 1 and exon 2 sequences. The canonical exons 1 and 2 are shown by boxes and the intron between them by a solid line. The GT and AG dinucleotides spanning the sequences that are used as introns in individual transcripts are indicated by small letters. G, Genomic structure, derived from sequencing segements of BAC clones encompassing the PTCH2 gene; C, Canonical transcript; A, Transcript A (the skipped exons 9 and 10 of this product are not shown in the diagram); B, Transcript B.

Figure 3A is a dark-field photomicrograph of a BCC tumor hybridised with ^{35}S-labeled antisense probe showing abundant signal for PTCH1 mRNA (light grains) in all BCC tumor cells.

Figure 3B discloses PTCH2 mRNA overexpression in BCC and is in contrast mainly expressed in the basaloid cells in the periphery of the tumor nests.

Figure 3C is another BCC showing a strong PTCH2 mRNA signal in the periphery of the tumor nest (Tu), wheras no signal is detected in epidermis (Ep).

Figure 3D are sections of the same tumor (C) hybridised with the PTCH2 sense probe showed no signal.

Figure 3E shows immunoreactivity for Ki-67 (brown precipitate) seen in the periphery, in the cells that showed strong upregulation of PTCH2 mRNA.

Figure 3F discloses tumor nests under high power magnification demonstrate abundant PATCH2 mRNA signal (black grains) in the dark basaloid tumor cells and lower signal in the center (arrow). Bars (A-E), 24 μm, and F, 6 μm.

EXPERIMENTAL

Materials and methods

In the present context, a general reference is made to G. Zaphiropoulos et al., Cancer Res., vol. 59, p. 787-792, February 15, 1999, disclosing useful methods in the present context. All references mentioned in the present application are hereby included herein by reference. The examples below are not intended to limit the scope of the invention but merely as an illustration.

The RACE analysis was performed essentially as described before (Zaphiropoulos, P.G. and Toftgård, R. (1996): "cDNA cloning of a novel WD repeat protein mapping to the 9q22.3 chromosomal region", DNA Cell Biol. **15**, 1049-1056) using the Marathon kit (Promega). The primer sequences used for RACE are available upon request.

The PTCH2, 35S-labeled RNA probes used for the in situ hybridisations, that were performed as previously described (Undén *et al.*, (1997), supra), corresponded to positions 218 to 437 and 838 to 920 in the PTCH2 sequence of SEQ ID NO:1 .

Results and discussion

In order to identify additional components of the PTCH/SHH cascade of signalling events, the Incyte LifeSeq™ database (Incyte Pharmaceuticals Inc., Palo Alto, CA, USA) was searched using PTCH sequences. In addition to clones representing the PTCH cDNA, two nearly identical cDNAs were identified, from the parotid gland and the colon, that contained sequences similar to, but distinct from, the 3' end of PTCH. By 5' RACE analysis using fetal brain cDNAs additional sequence information from these transcripts (termed PTCH2) and corresponding to a full length cDNA, was obtained (Fig. 2A). PTCH2 is 57% identical to PTCH1, with a significantly variable region present between the transmembrane domains 6 and 7, and 91% identical to the recently published mouse Ptch2 sequence (Motoyama, J., Takabatake, T., Takeshima, K. and Hui, C. (1998): "Ptch2, a second mouse Patched gene is co-expressed with Sonic hedgehog", Nature Genet. **18**, 104-106). In simila-

rity with the mouse gene, PTCH2 lacks the C-terminal extension present in human, mouse and chicken PTCH1 (Goodrich, L.V., Johnson, R.L., Milenkovic, L., McMahon, J.A., and Scott, M.P. (1996): "Conservation of the hedgehog/patched signalling pathway from flies to mice: Induction of a mouse patched gene by Hedgehog", Genes Dev. **10**, 301-312, Marigo, V., Scott, M.P., Johnson, R.L., Goodrich, L.V. and Tabin, C.J. (1996): "Conservation in hedgehog signalling: Induction of a chicken patched homolog by Sonic hedgehog in the developing limb", Development **122**, 1225-1233). However, according to the present invention, it has been shown that the human PTCH2 cDNA terminates 36 amino acids earlier that the mouse Ptch2 sequence. Moreover, when 3' RACE was perfomed from fetal brain, an alternate C-terminal region was identified. This had a high structural similarity with the mouse Ptch2 C-terminal sequence and originates from the genomic region that links the last two exons of PTCH2 (Fig. 2B). Therefore, in these alternatively spliced transcripts, the penultimate exon with a segment of the contiguous 3' intron serves as the terminal exon.

Moreover the human and mouse transcripts differed in the position of the termination signals (the human sequence is 21 amino acids longer), suggesting a non-conserved, species-specific function of this alternate C-terminal domain. The finding of two possible C-terminal regions for PTCH2 is intriguing and implies a role of this phenomenon in modulating signalling. Additional alternatively spliced transcripts were also identified by the RACE analysis (Fig. 2C). Transcript A lacks the sequence that corresponds to exons 9 and 10 of PTCH1 (preliminary comparisons of the intron-exon junctions of PTCH2 with PTCH1 indicate a similar genomic organization), with the open reading frame being retained at the exon 8 to exon 11 junction. Exons 9 and 10 code for the last part of the first extracellular loop and for transmembrane domains 2 and 3 in the putative structure of the PTCH1 protein. Furthermore this transcript also lacks a 5' segment of the canonical exon 2, due to the use of an alternative 3' splice site present in this exon, with the open reading frame being maintained. The functional consequence of this alternative splicing is not yet known, but it is interesting to note that the extracellular loops in PTCH1 are

presumed to be involved in binding of the ligand SHH (Marigo *et al.*, (1996), Nature **384**, supra; Stone *et al.*, (1996), Nature **384**, supra) and that insertion of a neocassette in intron 9 of of the mouse PTCH1 gene is associated with a severe phenotype (Hahn, H., Wojnowski, L., Zimmer, A.M., Hall, J., Miller, G. and Zimmer, A. (1998): "Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome", Nature Med. **4**, 619-622). Furthermore, exons 9 and 10 encode part of a putative sterol sensing domain (Osborne, T.F. and Rosenfeld, J.M. (1998): "Related membrane domains in proteins of sterol sensing and cell signalling provide a glimpse of treasures still buried within the dynamic realm of intracellular metabolic regulation", Curr. Opin. Lipidol. **9**, 137-140, also found in PTCH1, and which has recently been implicated in mediating the potent modulating effect of cholesterol on SHH/PTCH signalling (Cooper, M.K., Porter, J.A., Young, K.E., and Beachy, P.A. (1998): "Teratogen-mediated inhibition of target tissue response to Shh signalling", Science **280**, 1603-1607). Thus, if PTCH2 also serves as a receptor for SHH and/or related factors, the receptor form lacking exons 9 and 10 may show altered signalling properties. Transcript B contains additional sequences between canonical exons 1 and 2, that originate from the 5' end of intron 1. The open reading frame that includes the initiator methionine of exon 1 is not maintained in this transcript, suggesting that, if this transcript is functional, either the methionine in exon 2 or non-methionine codons are used in order to produce a protein product, in similarity to what has been proposed for the alternative spliced products of human PTCH1 (Hahn *et al.*, Cell **85**, supra). By radiation hybrid mapping the PTCH2 gene was localized to the short arm of chromosome 1, in difference to PTCH1 residing on chromosome 9q22.3.

The mouse and zebrafish homologs of PTCH2 have been reported to be expressed in a partly overlapping pattern with PTCH1 during embryonic development and to be induced by SHH (Motoyama *et al.*, (1998) Nature Genet. 18, supra, Concordet, J.P., Lewis, K.E., Moore, J.W., Goodrich, L.V., Johnson, R.L., Scott, M.P., and Ingham, P.W. (1996): "Spatial regulation of a zebrafish patched homologue reflects

the roles of sonic hedgehog and protein kinase A in a neural tube and somite patterning", Development **122**, 2835-2846), implicating a role in this signalling pathway. We were with this background interested to analyze the expression of PTCH2 in BCCs which show consistent upregulation of PTCH1 in all tumor cells (Undén *et al.*, (1997) Cancer res. **57**, supra). In situ hybridisation was performed on six familial and four sporadic BCCs of different histological subtypes. A strong positive signal for PTCH2 mRNA was observed exclusively in the tumor cells of all BCCs. Notably, the signal was consistently stronger in the palisading peripheral cells of the tumor nests (Fig. 2). These cells also showed a positive immunostaining for the cell proliferation marker, Ki-67.

The finding that in BCCs having frequent mutations in the PTCH1 gene, the expression of the PTCH2 mRNAs is upregulated, tightly links the novel PTCH2 according to the invention with the PTCH/SHH cascade of signalling events. It is therefore likely that PTCH2 represents a target gene of this pathway which is under the negative regulation of PTCH1, precisely as PTCH1 itself. Moreover this observation strongly suggests that PTCH2 has functions distinct from PTCH1 since upregulation of PTCH2 expression appears unable to compensate for inactive PTCH1 protein. This conclusion is also supported by the early embryonic lethality seen in PTCH1 (-/-) mice 5,13) and the lack of genetic heterogeneity in Gorlin syndrome. However, whether PTCH2 may block the constitutive signalling of SMO, or could act as an additional SHH receptor, possible dependent on alternative splicing, remains as the subject of further experimentation.

CLAIMS

1. An isolated human protein or an analogue or variant thereof capable of participating in the human PTCH/SHH pathway during embryonic development and/or carcinogenesis comprising at least about 1040 amino acids of SEQ ID NO: 1.
2. A protein according to claim 1, which is essentially comprised of SEQ ID NO: 1.
3. A nucleic acid encoding a protein according to any one of claims 1 and 2.
4. A nucleic acid encoding a protein according to claim 3 comprising at least about 3094 bases of SEQ ID NO: 2.
5. An isolated genomic nucleic acid comprising parts or all of SEQ ID NO: 5.
6. An isolated variant of the nucleic acid according to claim 5.
7. A nucleic acid having the sequence of a splicing variant selected from the group of variants defined in Figure 2B and SEQ ID NO:3 and SEQ ID NO:4.
8. An isolated nucleic acid capable of specifically hybridising to a nucleic acid according to any one of claims 3-7.
9. A protein or polypeptide encoded by a nucleic acid according to claim 7.
10. A protein according to claim 1 or 2, a nucleic acid according to any one of claims 2-8 or or a polypeptide according to claim 9, for use as a medicament.
11. Use of a protein according to claim 1 or 2, a nucleic acid according to any one of claims 2-8 or a polypeptide according to claim 9 in the manufacture of a medicament for the treatment of a condition involving tumors, such as BSS.
12. A method of <u>in vitro</u> or <u>in vivo</u> diagnosis, wherein a protein according to claim 1 or 2, a nucleic acid according to any one of claims 2-8 or a polypeptide according to claim 9 is used.
13. A method of screening wherein a library of suitable candidate compounds is screened for modified drugs using a protein according to claim 1 or 2 or a polypeptide according to claim 9 as a lead compound.
14. A method of synthesis of a modified drug, wherein a protein according to claim 1 or 2, or a protein or polypeptide according to claim 9, is used.
15. A modified drug identified by the method according to claim 13 or synthesized according to claim 14.

16. A vector comprising a nucleic acid according to any one of claim 3-8.
17. A recombinant cell comprising a vector according to claim 16.
18. An antibody which specifically binds to a protein or polypeptide according to claim 1, 2 or 9.
19. A recombinant cell expressing an antibody according to claim 18.
20. A kit for the detection of a human PTCH2 gene or polypeptide comprising in a container a molecule selected from the group consisting of a nucleic acid according to any one of claims 3-8, a polypeptide or protein according to claim 1, 2 or 9 or an antibody according to claim 18.
21. Use of a nucleic acid according to any one of claims 3-7 in gene therapy.
22. Use of nucleic acid according to any one of claims 3-7 as a probe, a primer or a diagnostic reagent.

The intron sequences between exons 2 - 3 and exons 18 - 19 are missing (introns: small letters, exons: capital letters). Small letters in the first exon indicate nucleotides that have not been unambigouisly determined.

Exon 1

```
   1  CGGGTGAATC CCGGCGCCGC GCCCCGGACC CGCAGCTCCC TGCACTCCTC
  51  CCTCCCAGCC GCTTTAACAC CCACACCCCA CAGTCTCTCC CACGsCCGCG
 101  CCTTGGCGGC CCCACTGAAT CCCTACGCGG GGCCCAGCGG TACCGGGAGA
 151  CCGGGCTAGC CTATGGGAGC GCCCAGATAA CGCGGGTTGG GGGCGCCCGC
 201  GCCCcCATCC CCGCCAGCAT GACTCGATCG CCGCCCCTCA GAGAGCTGCC
 251  CCCGAGTTAC ACACCCCCAG CTCGAACCGC AGCACCCCAG gtgagtagag
 301  ggggagctgg aagaaggaag agagcggagc caggtctgtc actcgggcct
 351  ctgcaaggtt tgtgatgtct tgaagtgccg agtgtcatta gatgtctgaa
 401  ggcaagtgag agccagcacc gcaagcaagt tgtgcgtgtg tgtcggtgtg
 451  tctgtgccgg tgtctcctca tcgtctggcc agtgagaatg aatgtctgtg
 501  ggttcacctc tgtgtccacc cgacgacagg tgtgtgtaca tatgtatcct
 551  gctctcagaa aatgggccta tgccgccggg cgcggtgact cacgcctgta
 601  atcccaacac tgggaggctg aggcaggcag attacctgag gtcaggagtt
 651  cgagaccagc caggccaaca tggggaaact ctgtctctac taaaaataaa
 701  aattagcagg gcgtggtggc gggcgcctgt agtcccaact actcgggagg
 751  ctgaggcagg agaatctctt gaacctggga ggcggaggtt gcagtcaagc
 801  cgagatcaca ccactgcact ccagccaggg caacagagcg agatgcgtct
 851  caaaaaaaaa aaaaaaaaaa aaaaggagag aaaacaaaaa gaaaagaaag
 901  gaaaataggc ctatgccttc ctcaggtgtg tgctggggat ggtgggtgtt
 951  acatcttcca agtctgggcc tgtgtctgtg ttggtgctcc ctgtcccaca
1001  tccagaaatc aagaagcgag ggctgggcag cagatataca gggtgagaag
```

Fig. 1

```
1051  ggaaggattt catgcattgt tacagtgatg cctggctgac ccttctcttt
                EXON 2
1101  ccatcccagA TCCTAGCTGG GAGCCTGAAG GCTCCACTCT GGCTTCGTGC
1151  TTACTTCCAG GGCCTGCTCT TCTCTCTGGG ATGCGGGATC CAGAGACATT
1201  GTGGCAAAGT GCTCTTTCTG GGACTGTTGG CCTTTGGGGC CCTGGCATTA
1251  GGTCTCCGCA TGGCCATTAT TGAGACAAAC TTGGAACAGC TCTGGGTAGA
1301  AGTGGGCAGC CGGGTGAGCC AGGAGCTGCA TTACACCAAG GAGAAGCTGG
1351  GGGAGGAGGC TGCATACACC TCTCAGATGC TGATACAGAC CGCACGCCAG
1401  GAGGGAGAGA ACATCCTCAC ACCCGAAGCA CTTGGCCTCC ACCTCCAGGC
1451  AGCCCTCACT GCCAGTAAAG TCCAAGTATC ACTCTATGGG AAG.......
1501  .......... .......... .......... .......... .........g
1551  tgagtctggc tgagcccctg agcagctggg ggcgaggcgt gctgtggggg
1601  ttctggagtg ggaatcccct tcttctgctg atctcctatg cccctggcta
          EXON 4
1651  ttgcagTCCT GGGATTTGAA CAAAATCTGC TACAAGTCAG GAGTTCCCCT
1701  TATTGAAAAT GGAATGATTG AGCGGgtaag tgtcctgaga gggagtagag
1751  gcagaacttt ttctgtagcg tgggaggact cagagaccga gcaagcccca
1801  cagcctgcaa tctgcccccc taaaactaag gaggggggatt gcagagggca
1851  tcctacaaag gttgtggggc aggactgacg tggcccgggg tatccctggc
        EXON 5
1901  agATGATTGA GAAGCTGTTT CCGTGCGTGA TCCTCACCCC CCTCGACTGC
1951  TTCTGGGAGG GAGCCAAACT CCAAGGGGGC TCCGCCTACC TGCCgtgagt
2001  gccactcctg gggccctgct tcatctcccg ctggggactc tcccagcaga
2051  aaggaggggt ctggggaatg aggatgatca aaaccttacc aaggtcctaa
2101  ttacctccca ggccaggaac agagagcatg ggcttcccca aggctctctc
2151  cacatcctcc ttctctttcc ctctcaagga aggaagacct gacttattta
2201  cacaaaacta aacacaaaga tctgtaagat ctgagcaaag gagaaaaaga
2251  tccccacaaa gaggctttgc tgggggaaat tacctaggtg tttgctaagc
2301  cattgcccag gccagaaaga aaacctgcta caggcatgtg cctgctggtt
2351  gtatattaga accaagcaca cagcttggta aggaactcag tggggccttt
```

Fig. 1 (cont.)

2401 ctgggccctt tctatgtatt aggtaaccct gccctgatat tcgtctcagc

2451 cccttgtact cttctacagc tcactgtagc accctggtgg gcccatgcag

2501 cctggcagtt ctgagaagct gaggcttgca caccctccat atggaaggac

2551 aaatcggcag ataagaggag ggtggggtac agcatggcgc cccagcagca

2601 gtttggagcc tgggttttcg tccctgaccc tcaccaacta taggcttttc

EXON 6

2651 cctcagCGGC CGCCCGGATA TCCAGTGGAC CAACCTGGAT CCAGAGCAGC

2701 TGCTGGAGGA GCTGGGTCCC TTTGCCTCCC TTGAGGGCTT CCGGGAGCTG

2751 CTAGACAAGG CACAGGTGGG CCAGGCCTAC GTGGGGCGGC CCTGTCTGCA

2801 CCCTGATGAC CTCCACTGCC CACCTAGTGC CCCCAACCAT CACAGCAGGC

2851 AGgtgggttc caaccaggtc tgccagggaa aggctgtttt ccttcccttt

2901 cccttcctca tactcctgtg ttctggggga gctgactgct ctgtgccctg

2951 acccccccact tcctggccat tattaccctg ctcccacagt gccaggcccc

3001 caatgttcca ttcccattca gttatcctac ggagccctca agtggtatat

3051 atgaatccct ttttcctttt ctaagcctag ataaggctgg acttcttttt

3101 ttttttttt ttgagtctca ctctgtcacc caggctggag tgcagtagtt

3151 cgatcttggc tcactgcaac ctcggctcaa gcaattctcc tgccttagcc

3201 tcctgagtag ctgggattac aggtgcccac caccatgccc ggctaatttt

3251 tattagcctc ccaaagtgct gggattacag gcgtgagcca ctgcgcctgg

3301 ccaaggctgg actttttatc aaaatagact aatacaggga aactaagaac

3351 acagcaggta agcatgaata tcatacctgg tttcccaggt ttctttgtgg

3401 ccctgcaaat gtggtacttt tttcagaatc cgccagttac accagctcct

3451 cccagaagcc tacttccagg cctctgcttc cccttggggc ttcctgtctg

3501 cgggatacta gctgttcact cctgcagagc agtcaagagg ctcagaatag

3551 ttacctacac tccagcccta ctgagcttca tggcagcgtg gttcctggag

3601 gtggaagccc agggacactc agttatccac ggccagggcc ttgagcatta

EXON 7

3651 acccctcctg ttcccctcca gGGCTCCCAA TGTGGCTCAC GAGCTGAGTG

3701 GGGGCTGCCA TGGCTTCTCC CACAAATTCA TGCACTGGCA GGAGGAATTG

Fig. 1 (cont.)

3751 CTGCTGGGAG GCATGGCCAG AGACCCCCAA GGAGAGCTGC TGAGgtaggg

3801 tctcctctgg gagttggtga ggggactctg ttcatgagaa cccatactgt

3851 aatgccaggc agctctggca aaaggccctt cacatccctc accaggtgtt

EXON 8

3901 tgggccagct ctgacccctg gttctcccac acccccacca gGGCAGAGGC

3951 CCTGCAGAGC ACCTTCTTGC TGATGAGTCC CCGCCAGCTG TACGAGCATT

4001 TCCGGGGTGA CTATCAGACA CATGACATTG GCTGGAGTGA GGAGCAGGCC

4051 AGCACAGTGC TACAAGCCTG GCAGCGGCGC TTTGTGCAGg tcggtatgga

4101 caaggacaag gggggtgccc tgaggccatt ccctcctcct gccccctcct

EXON 9

4151 atccaccctg tttctccagC TGGCCCAGGA GGCCCTGCCT GAGAACGCTT

4201 CCCAGCAGAT CCATGCCTTC TCCTCCACCA CCCTGGATGA CATCCTGCAT

4251 GCGTTCTCTG AAGTCAGTGC TGCCCGTGTG GTGGGAGGCT ATCTGCTCAT

4301 Ggtgggtctt gcacctggca ccttgccccc accccacctc caaccagtgc

EXON 10

4351 ccaccctggg agcccctgag actgcccttt ccccccacag CTGGCCTATG

4401 CCTGTGTGAC CATGCTGCGG TGGGACTGCG CCCAGTCCCA GGGTTCCGTG

4451 GGCCTTGCCG GGGTACTGCT GGTGGCCCTG GCGGTGGCCT CAGGCCTTGG

4501 GCTCTGTGCC CTGCTCGGCA TCACCTTCAA TGCTGCCACT ACCCAGgtac

4551 gccaggactg cagggcagac tcagtgccag tcaccaggct tcacgggtcc

EXON 11

4601 tcagctgccc gctcctctgc ccctccagGT GCTGCCCTTC TTGGCTCTGG

4651 GAATCGGCGT GGATGACGTA TTCCTGCTGG CGCATGCCTT CACAGAGGCT

4701 CTGCCTGGCA CCCCTCTCCA Ggtggggcct tgtcccccag ggctcatctg

4751 aggcagctca gcttactggt taagagcctc ttggttcaag tgacccttgg

4801 gctgctaatg aacctcggtg cctcttgtcc ccatctgtaa acaggggaaa

4851 taatagtgct gtgtcctaag ggttattgtt tggatcagtg aggtaactca

4901 agttgaatgc ttagaacagc ccatcatacg tacatggtac ccaataaatg

4951 ctagccactg tgttatgact gccccacctc tgcaccccaa gttcctgagc

5001 ctccccttca ctccactttg acacggcccc tcccttgtga cctgagggca

EXON 12

5051 ggtccccact ctgtcctggc agGAGCGCAT GGGCGAGTGT CTGCAGCGCA

Fig. 1 (cont.)

5101 CGGGCACCAG TGTCGTACTC ACATCCATCA ACAACATGGC CGCCTTCCTC

5151 ATGGCTGCCC TCGTTCCCAT CCCTGCGCTG CGAGCCTTCT CCCTACAGGC

5201 GGCCATAGTG GTTGGCTGCA CCTTTGTAGC CGTGATGCTT GTCTTCCCAG

5251 CCATCCTCAG CCTGGACCTA CGGCGGCGCC ACTGCCAGCG CCTTGATGTG

5301 CTCTGCTGCT TCTCCAGgta ctgcgtgcgc cccagcccct tcctcccgtg

5351 acccacgcca gcctgtcccc tcaccagcat ttcaaggcac agacctgtca

EXON 13

5401 tccactctct acctcttcca gTCCCTGCTC TGCTCAGGTG ATTCAGATCC

5451 TGCCCCAGGA GCTGGGGGAC GGGACAGTAC CAGTGGGCAT TGCCCACCTC

5501 ACTGCCACAG TTCAAGCCTT TACCCACTGT GAAGCCAGCA GCCAGCATGT

5551 GGTCACCATC CTGCCTCCCC AAGCCCACCT GGTGCCCCCA CCTTCTGACC

5601 CACTGGGCTC TGAGCTCTTC AGCCCTGGAG GGTCCACACG GGACCTTCTA

5651 GGCCAGGAGG AGGAGACAAG GCAGAAGGCA GCCTGCAAGT CCCTGCCCTG

5701 TGCCCGCTGG AATCTTGCCC ATTTCGCCCG CTATCAGTTT GCCCCGTTGC

5751 TGCTCCAGTC ACATGCTAAG gtaagactgg gcagagcagg gcagagactt

5801 agcatctctg ggcccagaag ggcagagagg gcttagtcca ctgcctgagg

EX

5851 ggctgggggc agccctgggg tctccagctt agttgctaca tcccgcagGC

XON 14

5901 CATCGTGCTG GTGCTCTTTG GTGCTCTTCT GGGCCTGAGC CTCTACGGAG

5951 CCACCTTGGT GCAAGACGGC CTGGCCCTGA CGGATGTGGT GCCTCGGGGC

6001 ACCAAGGAGC ATGCCTTCCT GAGCGCCCAG CTCAGGTACT TCTCCCTGTA

6051 CGAGGTGGCC CTGGTGACCC AGGGTGGCTT TGACTACGCC CACTCCCAAC

6101 GCGCCCTCTT TGATCTGCAC CAGCGCTTCA GTTCCCTCAA GGCGGTGCTG

6151 CCCCCACCGG CCACCCAGGC ACCCCGCACC TGGCTGCACT ATTACCGCAA

6201 CTGGCTACAG Ggtgagaggc gaggagacgg gcagggaggg gtgctgcagg

6251 gagaaacgcc ctggggccac cagctaatag aaccctatcc tggtctcccc

EXON 15

6301 cagGAATCCA GGCTGCCTTT GACCAGGACT GGGCTTCTGG GCGCATCACC

6351 CGCCACTCGA CCGCAATGGC TCTGAGGATG GGGCCCTGGC CTACAAGCTG

6401 CTCATCCAGA CTGGAGACGC CCAGGAGCTT CTGGATTTCA GCCAGgttgg

Fig. 1 (cont.)

```
6451  gagagggctg gaggggtcca ctagtacagg ggctgcaggc ctcctgggcc
                                        EXON 16
6501  caggccttca gccctctctg cctctgcagC TGACCACAAG GAAGCTGGTG
6551  GACAGAGAGG GACTGATTCC ACCCGAGCTC TTCTACATGG GGCTGACCGT
6601  GTGGGTGAGC AGTGACCCCC TGGGTCTGGC AGCCTCACAG GCCAACTTCT
6651  ACCCCCCACC TCCTGAATGG CTGCACGACA AATACGACAC CACGGGGGAG
6701  AACTTTCGCA gtgagtcttg gggggagctc ggcaagagcc tcagcctcgc
6751  ccacacaagc cctgagcctg aggccctgcc cactctgccc cgtgctcacc
                                                  EXON 17
6801  gccctgtccc tctccctctt ctcccttccc ctcccctcca cagTCCCGCC
6851  AGCTCAGCCC TTGGAGTTTG CCCAGTTCCC TTTCCTGCTG CGTGGCCTCC
6901  AGAAGACTGC AGACTTTGTG GAGGCCATCG AGGGGGCCCG GGCAGCATGC
6951  GCAGAGGCCG GCCAGGCTGG GGTGCACGCC TACCCCAGCG GCTCCCCCTT
7001  CCTCTTCTGG GAACAGTATC TGGGCCTGCG GCGCTGCTTC CTGCTGGCCG
7051  TCTGCATCCT GCTGGTGTGC ACTTTCCTCG TCTGTGCTCT GCTGCTCCTC
7101  AACCCCTGGA CGGCTGGCCT CATAgtgagt gcttgcagga gtggggacag
7151  agacacccca cccttccctg cccagcctgt catccctcct gccaggagcc
                                        EXON 18
7201  ctctgtgagc cctgtctccc tcagGTGCTG GTCCTGGCGA TGATGACAGT
7251  GGAACTCTTT GGTATCATGG GTTTCCTGGG CATCAAGCTG AGTGCCATCC
7301  CCGTGGTGAT CCTTGTGGCC TCTGTAGGCA TTGGCGTTGA GTTCACAGTC
7351  CACGTGGCTC TGGGCTTCCT GACCACCCAG GGCAGCCGGA ACCTGCGGGC
7401  CGCCCATGCC CTTGAGCACA CATTTGCCCC CGTGACCGAT GGGGCCATCT
7451  CCACATTGCT GGGTCTGCTC ATGCTTGCTG GTTCCCACTT TGACTTCATT
7501  GTAAG..... .......... .......... .......... ..........
7551  .......... gtagggaggg ctcggggcag ggaggcaggg ctcaggacag
                                                  EXON 20
7601  gcctgggctg actcccccca caccctaccc ctagGTACTT CTTTGCGGCG
7651  CTGACAGTGC TCACGCTCCT GGGCCTCCTC CATGGACTCG TGCTGCTGCC
7701  TGTGCTGCTG TCCATCCTGG GCCCGCCGCC AGAGgtgacc acaccctcgg
7751  caccatccct ctactcccag cccaagggac ggggtaggga gaggcaaggg
```

Fig. 1 (cont.)

7801 aagggacaga gccctgtggc ccacagacag gtacctcccc aacaggtgcc

7851 accagctgaa ggtggcagcc tcctcctttc cccagacacc atgttcctgc

7901 ccctcagccc tcctggcttc ttcatgggac ccaccttaga cttttaggat

7951 ccagaacaag gtgcagggtt tgccccaggc ctcaacatcc tgtcgcctgc

8001 cagctctcat atcctgctgg agaccaacaa gggccccagc ttcccaacag

8051 tcatggtaat ccccagcgag atgctaaagg ggacgggagc cccaggggcc

EXON 21

8101 cgtgggctta ctggggctgg tgtctcccca cagGTGATAC AGATGTACAA

8151 GGAAAGCCCA GAGATCCTGA GTCCACCAGC TCCACAGGGA GGCGGGCTTA

8201 Ggtgggggggc atcctcctcc ctgccccaga gctttgccag agtgactacc

8251 tccatgaccg tggccatcca cccacccccc ctgcctggtg cctacatcca

8301 tccagcccct gatgagcccc cttggtcccc tgctgtcact agctctggca

8351 acctcagttc caggggacca ggtccagcca ctgggtgaaa gagcagctga

8401 agcacagaga ccatgtgtgg ggcgtgtggg gtcactggga agcactgggt

8451 ctggtgttag acgcaggatg gacccctgga gggctctgct gctgctgcat

8501 cccctctccc gacccagctg tcatgggcct ccctgatatc catacagaac

8551 agccaccgat ttgcacatcc aggcctgtgt gagcctgtat ctgtgtcact

8601 tgagagtgaa agctggcact tggggctgca gtgcagccct gtccccttc

8651 ccaccccaca ccactgcctg cccagctgac caagcctgag ggaccctcca

8701 gcacccttcc gtctggtgac tcctgggcag gctctccata tccctgccca

8751 cctcctacca catccattat ttatatgaaa atgtctattt ttgtagtata

8801 catacatgtt agctatgatg aaagttttat tttttaaaga atgaaatata

8851 ttctatgtga agctatgatg aaagttttat tttttaaaga atgaaatata

8901 ttctatgtga actaatctcg aaagttttat tttttaaaga atgaaatata

8951 ttctatgtgt gcaagtgaac attagcttca gttgcttttt tttggacaga

9001 gtggggagtt tgcaagtgaa cattagctat tggaaggagc ttctctggtg

9051 ccaggacctg aggtattagc ttctctagtt ctgggtggaa aagaccccag

9101 attctggatt tttgtcatat acttggtaac atcatctgga ttaagtgctt

Fig. 1 (cont.)

9151 actatacaaa acgataacaa attttgttgg tgtgaaatcc tactgggttc

9201 aatctggaga ccgagagcag aaaaaaaaga accccactgt gtggctttca

9251 gagccaccat attccagcct gcccgtctct ccagactcac ctccacctac

9301 ctgcttcacc cgcacgggaa acggcaaggc agaggggcaa agccatgcag

9351 caggtggaag gcgaggtgga ggcagatcag gaaagcagcc agttgaagca

9401 gagagaggtc aacagggtct ggggagcttc tcaggaggtt tgtggaccca

9451 gggaaaggag ccaggttcca gagcaacctc caaggcaaag gcctctgtaa

9501 gttggttgtc ctgacagccg agaggtgtct ttggccagtc agccagtgga

9551 tcagttgcgg gaactgctca gaaactgagg tgctagcagt tagtgaggac

9601 acagcgtaag ttgtttgttc tgtgaaagtt gaacagctcc actaagcaga

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9751 catagtctag ttcacataga ccctgggctc caaccaccca ctcaccagga

9801 atgatcccac cccaggaaca atgcgttctc acatcccacc ccacctggac

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10001 ccagcaggcc caagggaggg aggctgcatt tgggtccagc agtgtttggg

10051 tcaccaagaa gggccttcta ggtggagcag agagagctca ccaggccaga

10101 atagtgcaaa gggggtcagc cctcagtgcc acttaccagc ggagtaaccc

E

10151 tgggcaagtt agccagcctc actaagcctc cccatcttca tctttccagG

XON 22

10201 CCCGAGGAGA TC**TAG**CCTCT GCCTCCCACC CCAGCACCCC CTCATCAGAC

10251 ACAAGGAGCG CCACTGTCTG GACAGGCTGA ATTGGTCTTC GGGTCCCTAA

10301 TTTCTCATAC GCCATTCCCT CTGCCTAGAA CACTTTCTCA CCTCCCCTTG

10351 ATGTGACCCC ATATCACCCT TCGAGGTGAA TTGGATCGGA TGCCATCTCC

10401 TCCAGGAGGG GTGGGGTCGT GCCTCCTGTG AGGTCCCAGT GCCCCTGAGT

10451 GTCTGTGCCC GTCTGTTTCC CCGTCCCTCT CTCTAAGCCC GGAGGCTTAC

Fig. 1 (cont.)

10501 TGCGGGTAAG GACGGCGGGA CAGGACCTTA ACCCCTGGGA CGAACACCAG

10551 CTCCGCAAAG GACTCCGCAC CCGGCGCCGC CCACGGGGTG CGGGTCCCAG

10601 GAGGACCAGC AGAGAGGAGC ATAGGAGAGC AAAGGAGATC AGTGACCCAT

10651 GGCTTCCCCG GTGGCGCGGA ACAGCCCGGA GCCGCCTGTG ATTTGCATAC

10701 CCATGGTGCA CCACGAAAAG ATACCCTCAA GATGCTTGCA CTCCCTCTGT

10751 GCGCGCATTT CTGCACTGTT TTAGAGCATG ATGCCTCTTA CACGCATCTG

10801 TGTGCATAAA CTACATATAG GGAGTGCGTA CCACGCAGGC ATCCAACAAC

10851 CATAAGTGTG TTAAGTGTTA GTTCTCCCTG CGAGGTTCGA AGCGGAAGTC

10901 ACGAATATAC TCGGGTTTCT CTTCAAAGCG CATAAATCTT TCGCCTTTTA

10951 CTAAAGATTT CCGTGGAGAG AAAGTTGTGA GTTTTTATTC AATTTTTTGA

11001 GGCCTCTTAT TTCCTGAGGC TACATTTTTA AGTATTAAAA GTTAGGCAAC

11051 TACAAAAAAA AAAAAAAA

Fig. 1 (cont.)

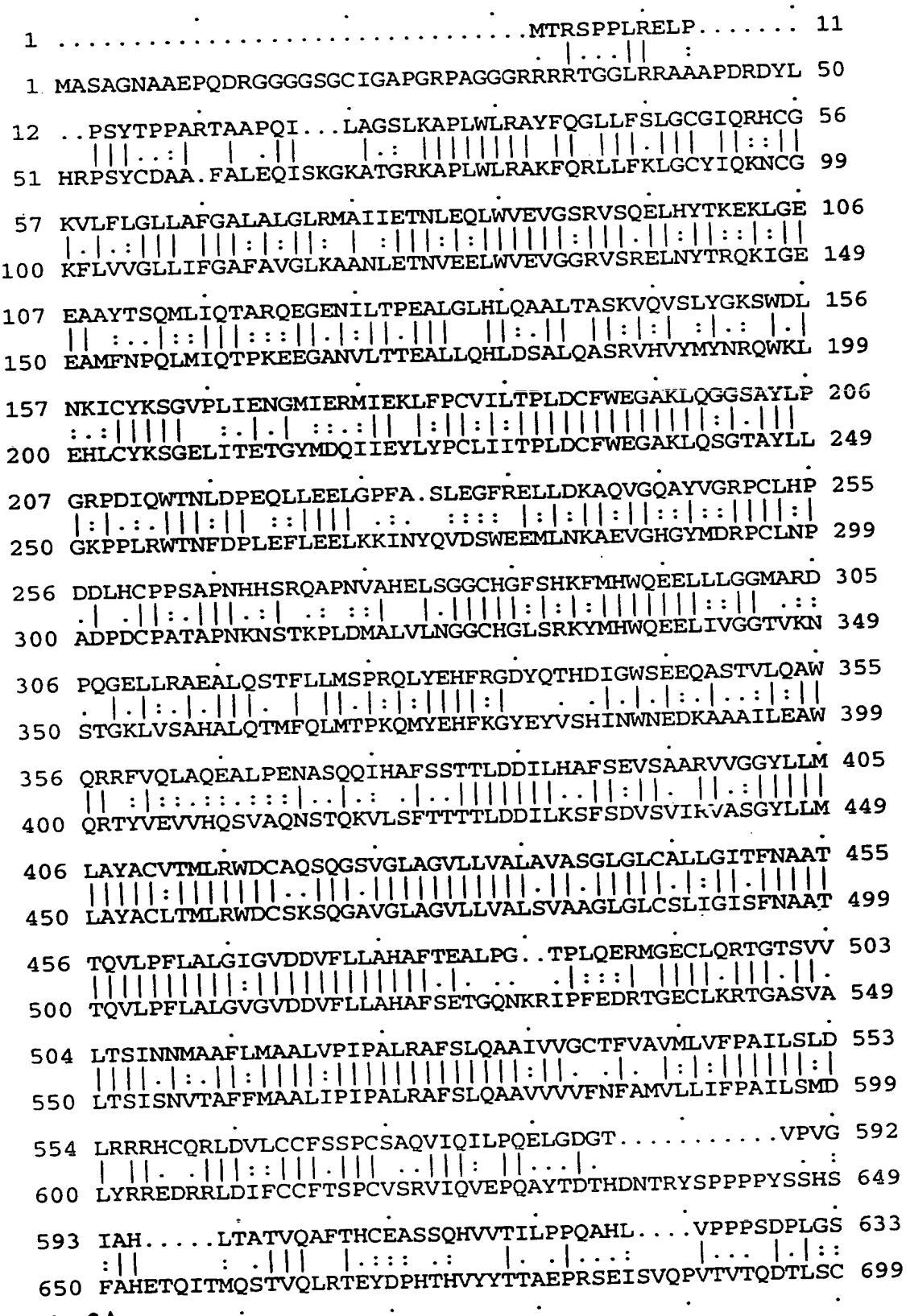
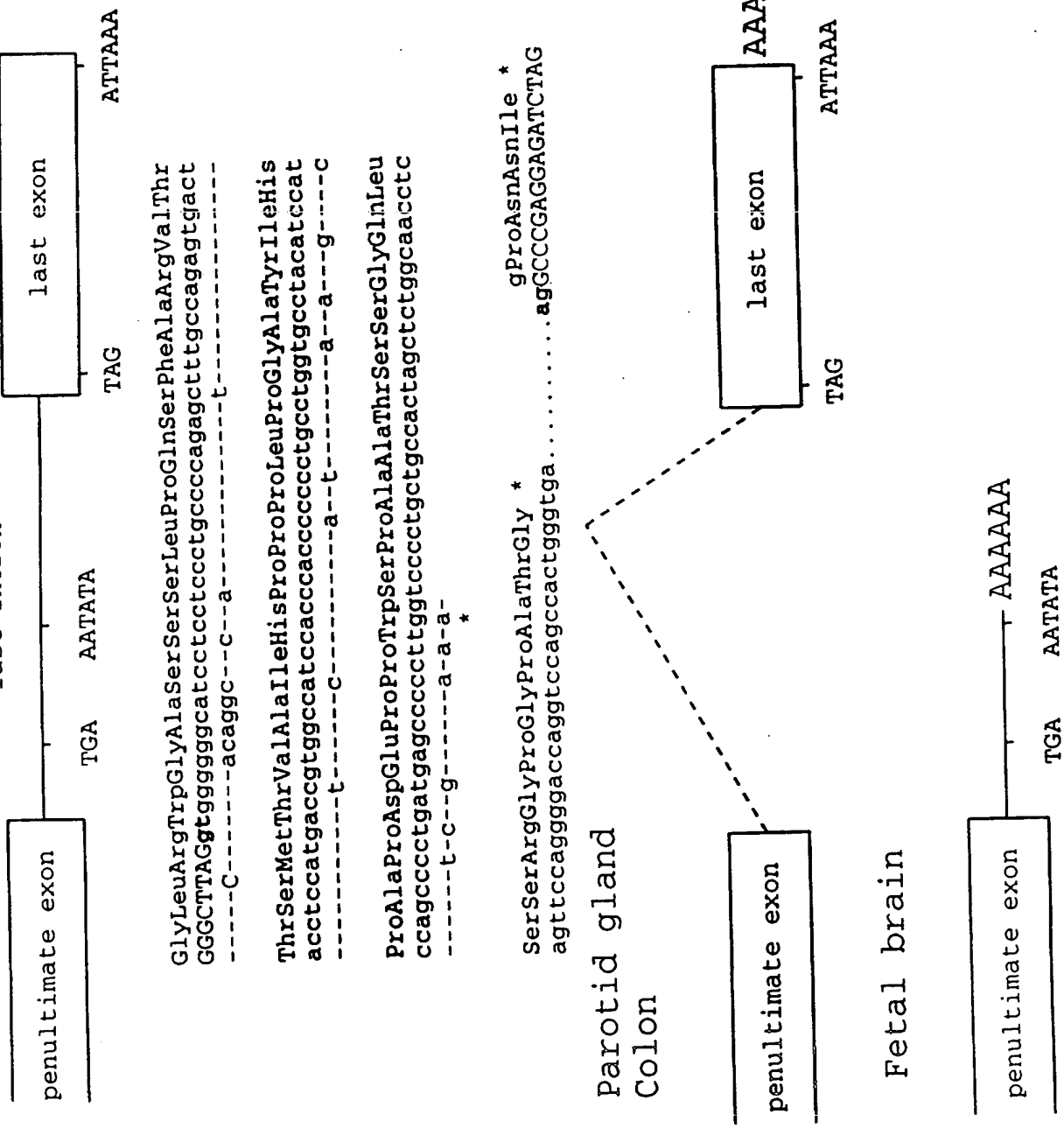


Fig. 2A

Fig. 2B


Genomic
last intron
penultimate exon
TGA
AATATA
last exon
TAG
ATTAAA
GlyLeuArgTrpGlyAlaSerSerSerLeuProGlnSerPheAlaArgValThr
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ThrSerMetThrValAlaIleHisProProProLeuProGlyAlaTyrIleHis
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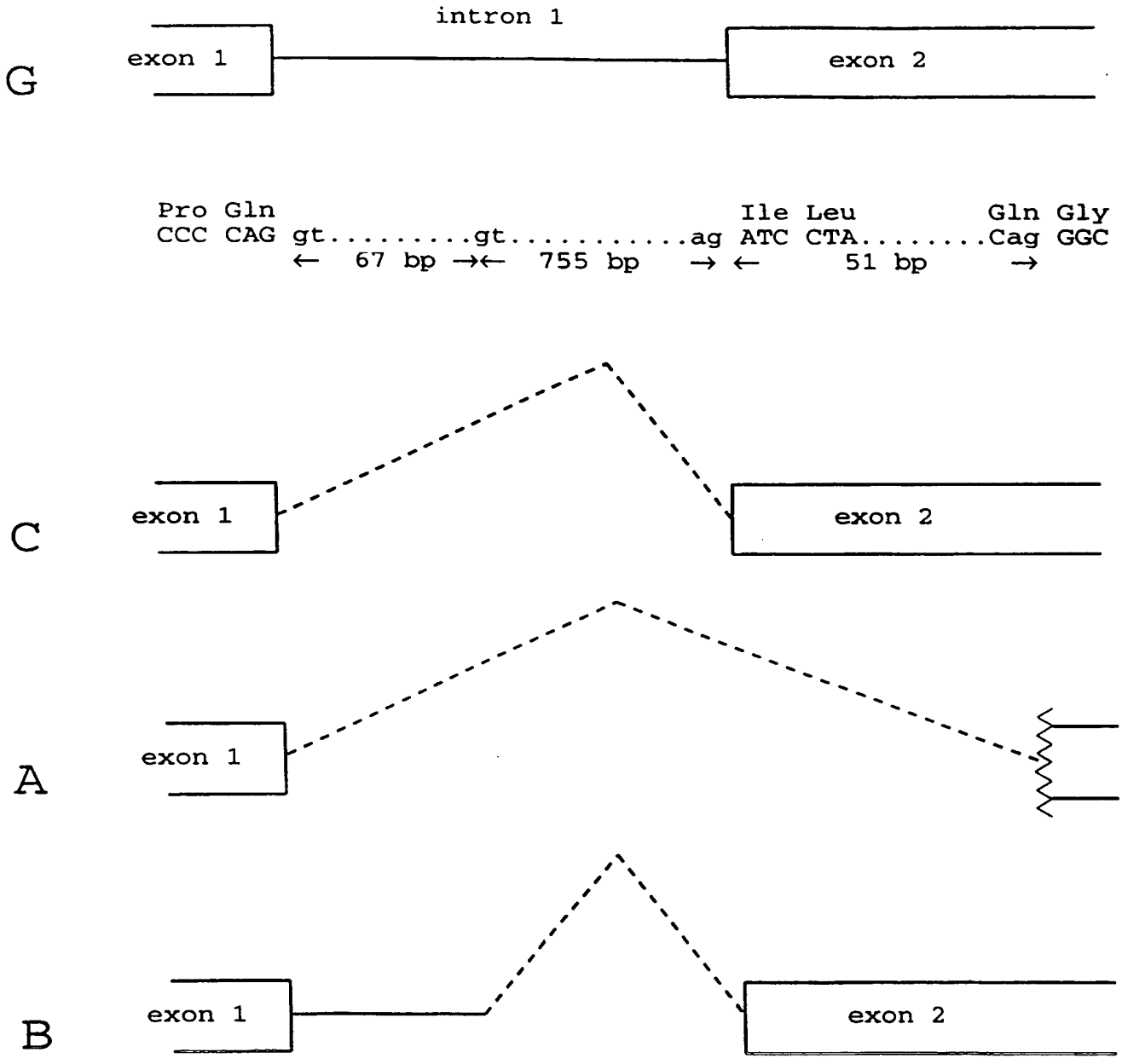
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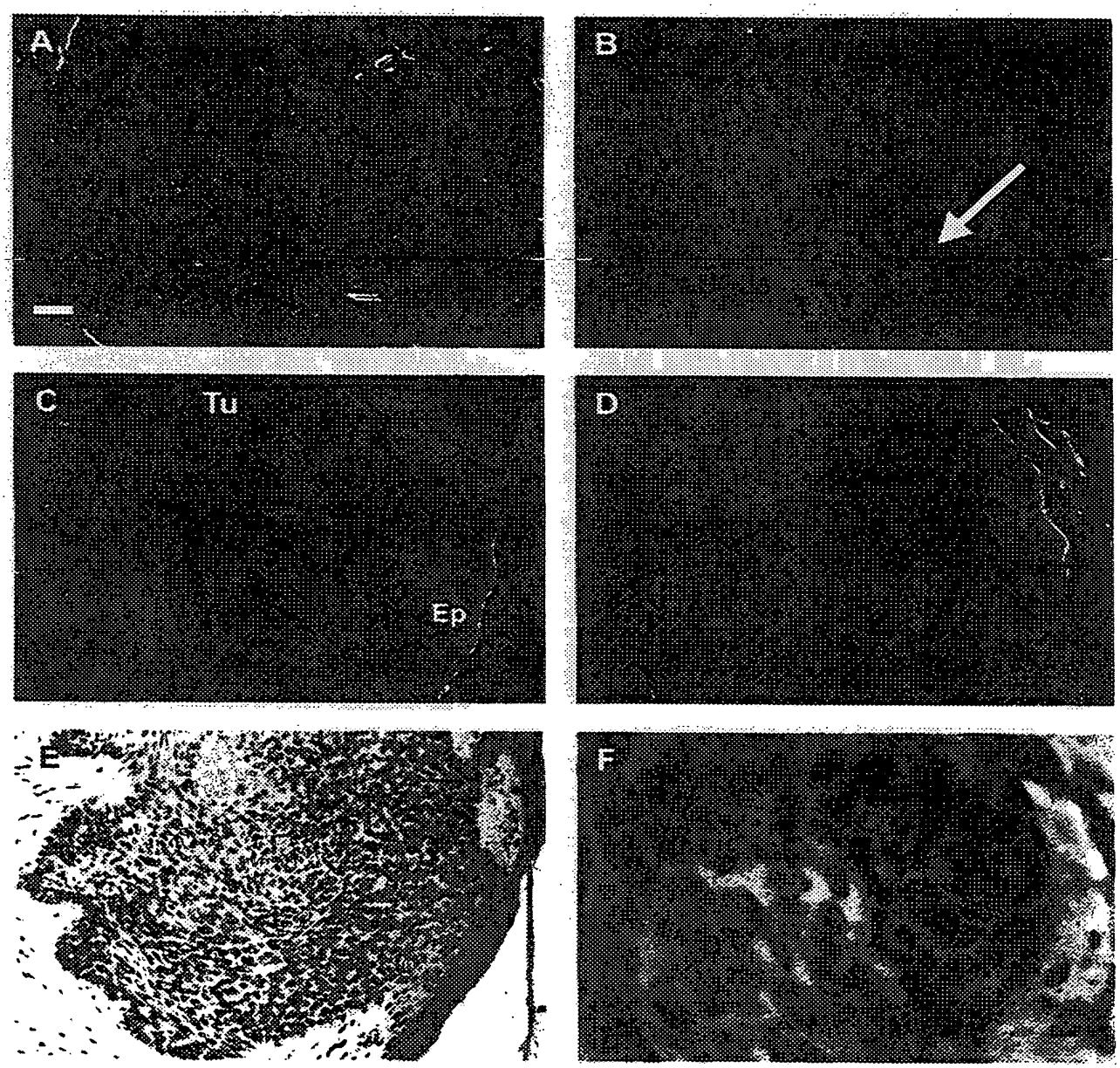

Fig. 3

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KAROLINSKA INNOVATIONS AB

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01784

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 48/00, C07K 14/705, A61K 38/17, G01N 33/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, C07K, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9929854 A1 (ONTOGENY, INC.), 17 June 1999 (17.06.99), page 73 - page 77, claim 3 --	1-12,16-22
P,X	EP 0879888 A2 (SMITHKLINE BEECHAM PLC), 25 November 1998 (25.11.98), claim 9b --	1-12,16-22
P,X	Journal of Clinical Investigation Online, Volume 95, No 23, November 1998, David Carpenter et al., "Characterization of two patched receptors for the vertebrate hedgehog protein family", pages 13630-13634 --	1-12,16-22

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 February 2000	14 -02- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson/AE Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01784

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Nature Genetics, Volume 18, February 1998, Motoyama J. et al., "Ptch2, a second mouse Patched gene is co-expressed with Sonic hedgehog", page 104 - page 106, figure 1, EMBL Acc no AB000847	1-12,16-22
A	FEBS Letters, Volume 410, 1997, Takashi Takabatake et al., "Hedgehog and patched gene expression in adult ocular tissues" page 485 - page 489	1-12,16-22
A	WO 9745541 A2 (THE LELAND S. STANFORD JUNIOR UNIVERSITY), 4 December 1997 (04.12.97)	1-12,16-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE99/01784

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.: claims 12 partially and claim 21 completely
because they relate to subject matter not required to be searched by this Authority, namely:

See next sheet.

2. [x] Claims Nos.: 13-15
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See next sheet.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE99/01784

Box I. 1.

Claims 12 partially 21 completely relate to methods of treatment of the human or animal body by therapy/ diagnostic methods practised on the human or animal body See PCT Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds.

Box I. 2.

Claim 13 relates to a screening method, it is not clear from this claim or the description how the polypeptide is used to achieve the screening. Claim 14 relates to a method of synthesis a modified drug, it is not clear from the description or the claim how the protein is used. Claim 15 relates to a drug identified by any of the methods in 13 or 14. Thus, claims 13-15 is considered to fail to comply with PCT-article 6 and have not been searched. Moreover, claim 15 concerns a drug per se, the drug may be a known substance. The description or the claims does not give any indication on that any unknown substance(s) that are preferred. For this reason, a search or a statement concerning novelty and an inventive step can not be made for claim 15.

Claims 12 partially 21 completely relate to methods of treatment of the human or animal body by therapy/ diagnostic methods practised on the human or animal body See PCT Rule. 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds.

Claim 13 relates to a screening method, it is not clear from this claim or the description how the polypeptide is used to achieve the screening. Claim 14 relates to a method of synthesis a modified drug, it is not clear from the description or the claim how the protein is used. Claim 15 relates to a drug identified by any of the methods in 13 or 14. Thus, claims 13-15 is considered to fail to comply with PCT-article 6 and have not been searched. Moreover, claim 15 concerns a drug per se, the drug may be a known substance. The description or the claims does not give any indication on that any unknown substance(s) that are preferred. For this reason, a search or a statement concerning novelty and an inventive step can not be made for claim 15.

INTERNATIONAL SEARCH REPORT
Information on patent family members

02/12/99

International application No.
PCT/SE 99/01784

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9929854 A1	17/06/99	AU 1716599 A	28/06/99
EP 0879888 A2	25/11/98	CA 2232808 A	23/11/98
		GB 9710752 D	00/00/00
		JP 11075874 A	23/03/99
		GB 9805954 D	00/00/00
WO 9745541 A2	04/12/97	AU 3227497 A	05/01/98